

Morphological characterization and quality evaluation of some cultivated paprika morphotypes (*Capsicum annuum* L.) from Tadla-Azilal region of Morocco

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Abstract

To characterize eleven different morphological forms of Moroccan paprika grown under field conditions in Tadla-Azilal region, analysis of morphometric data of different morphotypes (variants) was performed. The 11 morphotypes were evaluated for their qualitative traits of ripe fruits. Statistically significant differences among these variants were found for all the fruit characters studied. The evaluated morphotypes differed also in vitamin C, capsaicin, ASTA value and coordinated chromatic of color. The morphotype fruits evaluated had high genetic diversity and potential to fulfill the industry requirements. Morphotype 1 had the most desired commercial trait such as high ASTA value, high DW/FW ratio and low pungency. The results obtained in this study can be used as accurate information to establish a program of breeding to develop new commercial hybrids with fruits enriched for more desired commercial traits.

Key words: Paprika, Morphological traits, commercial traits, Vitamin C, ASTA; Carotenoids, Capsaicinoids.

1. Introduction

The *Capsicum* genus comprises a highly diverse group of sweet and hot peppers that are originated from the tropics of the American continent (Paik et al. 2003). It comprises more than 200 varieties (Pruthi 1980) which the most cultivated species are *Capsicum annuum*, *Capsicum frutescens*, *Capsicum chinense*, *Capsicum baccatum* and *Capsicum pubescens* (Pruthi 1980). *Capsicum annuum* is the most diverse and cultivated pepper, and is comprised of both sweet and hot peppers varieties.

In Morocco, sweet pepper (*Capsicum annuum* L.) called Niora is used for making paprika. The main production area is Tadla Region with more than 80% of the national production (Hakmaoui et al. 2011).

Pepper fruits vary in size and shape and contain a broad variety of carotenoids, flavonoids, phenols, ascorbic acid, capsaicin, and other components, which determine the great variability of the fruit's smell, flavor, taste and consequently consumer preference. The fruit quality and composition change according to the ripeness stage (Navarro et al. 2006; Conforti et al. 2007; Deepa et al. 2007), and the environmental conditions in which the fruit was grown and in the case of cultivated varieties, the crop management (Medina-Lara et al. 2008; Monforte-González et al. 2010).

The physical appearance and presentation of paprika, like most foodstuffs, significantly influence a prospective consumer's sensory evaluation and play a prominent role in final selection and consumption (Clydesdale 1993). The color of paprika is very important and is the principal criterion for assessing its quality and value, because its color largely determines the price which a producer receives (Minguez-Mosquera et al. 1992). The color of paprika mainly comes from the carotenoids formed in the fruit during ripening, with more than 30 different pigments identified (Deli et al. 2002; Topuz et al. 2009).

The high concentration of the antioxidant ascorbic acid in paprika plays a positive role in ensuring the stability of the final product (Fernández-Trujillo & Escarabajal 2006). Pungency, a commercially important attribute of paprika, is due to the presence of alkaloid compounds in the fruit known as capsaicinoids (Hoffman et al. 1983; Zaki et al. 2013). The two most abundant capsaicinoids in pepper are capsaicin and dihydrocapsaicin, accounting for 69 and 22% respectively of the total capsaicinoids in most of the pungent varieties (Kosuge & Furuta 1970). Usually, sweet paprika, the most commonly grown pepper in Morocco and Spain, has very low capsaicinoid content (Fernández-Trujillo & Escarabajal 2006).

Because of its popularity in the Tadla-Azilal region of Morocco (Hakmaoui et al. 2011; Zaki et al. 2013), *Capsicum* "Niora" has been the main focus of researchers. Nevertheless, little is known about the composition of the fruits; neither the accession, nor the landraces of those which are cultivated and gathered in the region of study. In addition, the expansion of the crop is limited by some factors, such as the lack of clear differentiation among varieties, low fruit quality (heterogeneity and phytosanitary problems), use of inappropriate varieties, or substitution of local varieties with materials from other origins. As a first measure to improve the quality factors of cultivars, it is necessary to screen existing cultivars, in order to start a program of breeding. Therefore, the

purpose of this study was to determine the morphological and the chemical diversity of eleven morphotypes of fruits of paprika cultivated in Tadla-Azilal region.

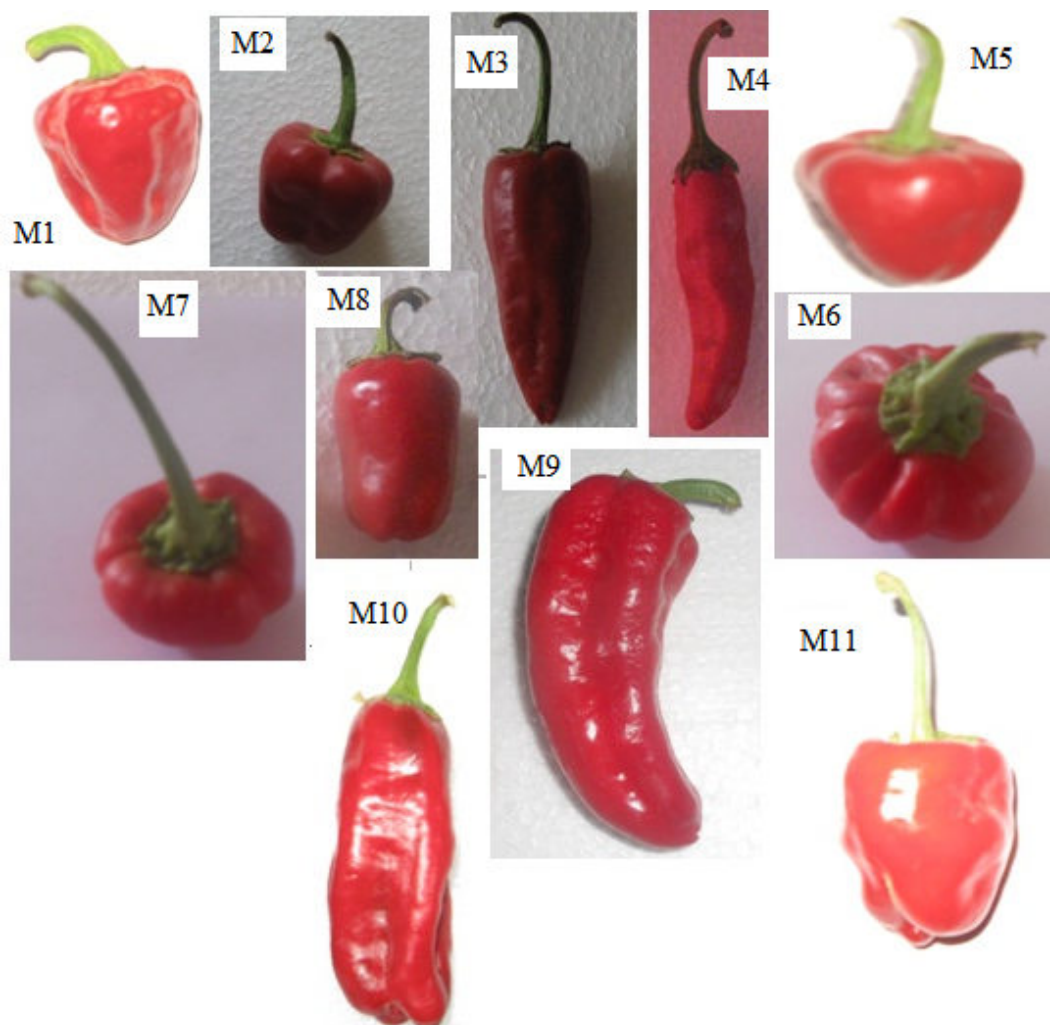


Figure1: Morphotypes of *Niora capsicum annuum L.* collected from Tadla-Azilal area.

2. Materiel & methods

Based on preliminary investigation, we could differentiate in drying site and in fields of cultivated niora many morphotypes from which 11 are characterized clearly by high morphological variability (figure 1). The fruits were collected in a fresh state from the fields at the middle of September 2011 at the first harvest. The entire sample was dried at 55 °C in a forced air-flow oven.

2.1. Morphological traits measurement

A sample of 10 ripe fruits of each morphotype was used to measure the morphological traits such as fruit length, fresh and dry weight fruit, length of pedicel, fruit density, seeds number and weight and percentage of dry seed to entire fruit dry weight. The fresh samples were dried at 55 °C in a forced air-flow oven during 48h until dry weight stabilized to determine dry weights percent.

2.2. Measurement of paprika color and chemical composition

Powder derived from the eleven paprika fruit collected was used for this investigation. Only the pedicels were removed and the entire sample was dried at 55 °C in a forced air-flow oven and was ground together. The average particle size of the powders was 400 µm. The samples were stocked in hermetic containers in darkness and under refrigeration (4 °C) until analysis.

The ASTA color value of paprika powders was determined according to the official AOAC method 971.26 (Horwitz 2002) with a slight modification. Samples (0.1 g) were extracted with 20 ml acetone for 3 h by using a water bath (axially shaken at 140 rpm) maintained at 25 °C. Then the extract was diluted 1/5 with acetone. The absorbance of the diluted extract was measured against acetone at 460 nm by spectrophotometer. The extractable

color of the samples was expressed in ASTA units:

$$\text{ASTA} = \text{Absorbance} \times 16.4 \times \text{devf} / \text{weight},$$

where devf is the deviation factor of the spectrophotometer, which was calculated by dividing the theoretical absorbance by the real absorbance of standard color solution (0.001 M $\text{K}_2\text{Cr}_2\text{O}_7$ and 0.09 M $(\text{NH}_4)_2\text{Co}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ in 1.8M H_2SO_4) at 460 nm.

Chemical color determination (Tint) was evaluated by dividing the absorption at 470 nm by the absorption at 455 nm of the acetone extracts (De Guevara & Pardo 1996; Hornero-Méndez & Mínguez-Mosquera 2001).

To measure skin color, a CR-300 chromameter (Minolta, Osaka, Japan) was used for (C illuminant, 0° viewing) previously calibrating it with a white-plate standard. A glass Petri dish containing the samples was placed below the light source. The L, a, and b values of each sample were determined in triplicate. Because chroma (C) and Hue angle (h°) have been shown to be more practical measures of color from an human sensorial point of view (Mcguire 1992), both parameters were calculated: $C = (a^2 + b^2)^{1/2}$ and $h^\circ = \arctan(b/a)$.

The capsinoid content was determined as described by Collins et al. (1995) and the results were converted to the Scoville Heat Units (SHU) by multiplying the individual capsinoid composition (in mg kg^{-1} dry weight of the paprika) by the coefficient of the heat value for each individual compound, 9.3 for nordihydrocapsaicin (NDHCAPS) and 16.1 for both capsaicin (CAPS) and dihydrocapsaicin (DHPS) (Todd et al. 1977).

$$\text{Total SHU} = [\text{CAPS} + \text{DHCAPS}] \times 16.1 + [\text{NDHCAPS} \times 9.3]$$

Total carotenoids content was determined by the method of Ala-salvar et al. (2005) with slight modification. In brief, dried samples (0,500 g) were extracted with 5 ml of acetone–water (9:1, v/v) and centrifuged at 3000 rpm for 10 min at 4°C . The clear supernatant was withdrawn and extraction was repeated for another five or six times with 3 ml of acetone–water until no colour was extracted. Extracts obtained were pooled and measured against an acetone blank at 471 nm using a UV-2100 spectrophotometer.

The vitamin C was determined using the method of Benderitter et al. (1998).

2.3. Statistical analysis

Analysis of variance of the data from each attribute was computed using the SPSS logiciel software version 10.0. When the effect of morphotype was significant, the Duncan test at 5% level of probability was used to test the differences among mean values.

3. Results

3.1. Morphological parameters

The eleven morphotypes of “Niora” (*Capsicum annum L.*) were significantly different ($P < 0.05$) in fruit characteristics such as fresh and dried fruit weight, fruit length, , number of seeds per fruit, seed weight, fruits density, and pedicel length (Table1). The average fresh fruit weight ranged from 5.14 to 26.23 g with M 8 and M 10 having significantly lower and higher fresh fruit weight, respectively. For DW/FW ratio, the highest (25.29%) and lowest (15%) one were observed in M 8 and M10, respectively. Number of seeds per pod ranged between 114 to 266, highest being in M11 and lowest in M4, M6 and M8. Percent of seed in dry weight varied between 14.16 to 43.69%. Maximum was noticed in M7 and minimum in M10. The length of pedicel varied from 1.96 to 5.73 cm. the highest was found in M7 and lowest in M8. The flavor of the final product may be changed if during the milling process of obtaining pepper powder, calyx and pedicel were added (Casali & Stringheta 1984). The fruits of M6 showed the highest 100 seeds weight (0.759g). The fruits of M2 and M11 showed the highest number of seeds.

Table 1: Morphological characteristics of fruits morphotypes collected from Tadla-Azilal area, Morocco.

Parameters	Fresh weight (g)	DW/FW (%)	Density (g /cm ³)	Pedicle length (cm)	Fruit length (cm)	Seeds per fruit (%)	Number of seeds per fruit	Weight of 100 seeds (g)
Morphotype								
M1	11.42±3.32 ^{de}	23.52±2.99 ^{ab}	0.54±0.07 ^{bc}	3.04±0.47 ^{def}	4.31±0.89 ^{cd}	39.49±6.41 ^{ab}	180±56 ^{bcd}	0.645±0.07 ^{bc}
M2	18.35±4.59 ^{bc}	20.53±3.08 ^{cd}	0.51±0.04 ^c	3.25±0.54 ^{cde}	3.20±0.35 ^{de}	36.25±1.27 ^{abc}	230±58 ^{ab}	0.566±0.09 ^{bcd}
M3	9.43±2.68 ^e	19.61±3.44 ^d	0.38±0.04 ^e	3.73±0.84 ^c	6.10±0.31 ^b	37.56±7.28 ^{abc}	133±49 ^{de}	0.541±0.15 ^{de}
M4	9.39±2.27 ^e	18.65±1.73 ^d	0.49±0.05 ^{cd}	2.86±0.59 ^{ef}	6.25±0.25 ^b	36.80±5.48 ^{abc}	115±37 ^c	0.566±0.06 ^{bcd}
M5	14.54±5.52 ^{cd}	19.46±3.45 ^d	0.59±0.10 ^{ab}	2.65±0.68 ^f	2.47±0.24 ^{ef}	33.73±10.6 ^{bc}	197±72 ^{bc}	0.625±0.14 ^{bcd}
M6	15.53±5.52 ^{cd}	17.58±2.85 ^d	0.55±0.08 ^{abc}	2.50±0.58 ^f	2.35±0.14 ^f	31.79±7.24 ^c	114±60 ^c	0.759±0.08 ^a
M7	13.16±3.17 ^{de}	22.64±3.52 ^{bc}	0.62±0.09 ^a	5.73±0.73 ^a	1.95±0.25 ^e	43.69±5.94 ^a	198±48 ^{bc}	0.658±0.12 ^b
M8	5.14±0.56 ^f	25.69±2.42 ^a	0.61±0.03 ^a	1.96±0.24 ^g	3.05±0.22 ^{de}	38.81±3.21 ^{abc}	118±19 ^c	0.436±0.04 ^f
M9	12.13±4.02 ^{de}	20.11±5.07 ^{cd}	0.49±0.08 ^d	3.50±0.85 ^{cd}	5.50±0.77 ^{bc}	34.56±10.1 ^{bc}	155±52 ^{cde}	0.569±0.03 ^{bcd}
M10	26.23±6.79 ^a	15.00±3.89 ^e	0.53±0.04 ^{bc}	2.83±0.39 ^{ef}	8.25±0.34 ^a	14.16±7.08 ^d	132±78 ^{de}	0.482±0.03 ^{ef}
M11	19.62±2.22 ^b	19.47±0.97 ^d	0.53±0.04 ^{bc}	4.69±0.37 ^b	4.12±0.51 ^{cd}	37.71±3.08 ^{abc}	266±48 ^a	0.548±0.06 ^{cde}
ANOVA	***	***	***	***	***	***	***	***

Values are mean ± SD of 3 replicates. Means within the same row carrying different superscript letter were significantly different at $p < 0.05$ according to a Duncan multiple range test

In this study statistically significant differences were found for most traits, which indicate that a wide variation exists and that it is possible to select materials with fruit characteristics more appropriate for the market. The commercial value of paprika fruits depends primarily on its content of dry matter. The high content of dry weight was recorded in M8 (26%) and M1 (24%). The DW/FW ratio is oppositely related to the fruit weight, so the longer is the fruit, the lower is its DW/FW ratio (data not shown). Dry matter content is an important trait for breeding Capsicum for industry, since the higher dry matter content per fruit, the higher the yield in the use of dry or powder of chili and peppers (Lannes et al., 2007). The variations in fruit dry weight among varieties may be due to the genetic and to the agro-ecological variations in which the varieties were evaluated. Guerpinar & Mordogan (2002) reported that pod dry matter content of peppers was directly related to the amount of nutrient taken from the soil, which was proportional to the nutrients present in the soil or the amount of organic and inorganic fertilizers applied to the soil.

3.2. Color of paprika

The color of paprika powder is measured either as extractable red color (Figure 2A) or surface color (Table 2). Extractable color is the official method used by the American Spice Trade Association (ASTA 1999) and in international trade. The ASTA extractable color results reflected in figure 1A showed significant differences between morphotypes. The ASTA values ranged from 170 (M1) to 80 (M10). Only M7 and M10 had ASTA value below 100 ASTA units.

The values of reflected color parameters are presented in table 2, as means with standard errors. Visual color of the samples investigated was measured by coordinates CIE Lab L* (lightness), a* (redness) and b* (yellowness) and estimators Hue (h°) and chroma (C). The values of these coordinates varied between morphotypes. The L* values varied in a wide interval, which indicates that color brightness also differed between hybrids. It varied from 24.37 to 29.75 respectively in M2 and M1. The value of a* varied from 25.59 (M7) to 34.55 (M8) and the b* value varied from 42.03 (M2) to 50.75 (M8). For the value of chroma (C), the morphotype M9 had the lowest (50.88) and M8 had the highest (61.39) value. The Hue (h°) value varied slightly among morphotypes from 0.97 (M8) to 1.08 (M7).

Table 2: Chromatic coordinates (L^* , b^* , a^*), C, h° for the different morphotypes samples

Morphotypes	L^*	a^*	b^*	C	h°
M1	29.75±0.12 ^a	32.61±0.09 ^a	49.75±0.06 ^a	59.49±0.26 ^a	0.99±0.05 ^a
M2	24.37±0.21 ^c	28.97±0.14 ^b	42.03±0.13 ^c	51.05±0.13 ^{bc}	0.97±0.11 ^a
M3	28.55±0.15 ^a	28.56±0.27 ^b	48.27±0.26 ^{ab}	56.09±0.25 ^{ab}	1.04±0.08 ^a
M4	25.68±0.19 ^a	26.89±0.43 ^c	44.28±0.23 ^{bc}	51.81±0.19 ^{bc}	1.03±0.06 ^a
M5	26.17±0.22 ^{bc}	29.41±0.28 ^{ab}	45.14±0.18 ^{bc}	53.88±0.37 ^b	0.99±0.02 ^a
M6	25.98±0.32 ^{bc}	27.21±0.16 ^{bc}	44.81±0.22 ^{bc}	52.42±0.22 ^{bc}	1.03±0.06 ^a
M7	27.87±0.20 ^{ab}	25.59±0.27 ^c	47.44±0.32 ^{ab}	53.90±0.45 ^b	1.08±0.03 ^a
M8	29.74±0.18 ^a	34.55±0.35 ^a	50.75±0.08 ^a	61.39±0.27 ^a	0.97±0.03 ^a
M9	24.72±0.14 ^c	27.78±0.15 ^{bc}	42.63±0.10 ^c	50.88±0.36 ^{bc}	0.99±0.07 ^a
M10	27.46±0.41 ^{ab}	27.53±0.32 ^{bc}	46.67±0.39 ^b	54.18±0.17 ^b	1.04±0.02 ^a
M11	26.9±0.26 ^b	29.05±0.25 ^{ab}	46.39±0.19 ^b	54.74±0.22 ^b	1.01±0.04 ^a

Values are mean ± SD of 3 replicates. Means within the same row carrying different superscript letter were significantly different at $p < 0.05$ according to a Duncan multiple range test

For the tint, the ratio Red/Yellow values averaged from 0.96-0.99 without any significant difference between paprika fruits morphotypes (Fig. 2D). Carvajal et al. (1997) and Topuz et al. (2009) reported similar R/Y values in paprika.

The carotenoids contents varied significantly among morphotype (Fig 2B). The highest and lowest values were found in M1 (3807 mg kg⁻¹ DW) and M10 (1796 mg kg⁻¹ DW), respectively.

Surface color measurements will give some indication as to how the paprika powder will look to the eye. As it is difficult to interpret complex ' L ' and ' h° ' data, the standard technique used by the spice industry is to measure extractable color and to observe the powder visually for defects. The ASTA method is the most widely used to measure the commercial quality of paprika. This quantifies the total carotenoid content indirectly, and has been used as a parameter of quality in selection, breeding, and cultivar characterization work (Costa et al. 2001; Kim et al. 2002). M7 presented the lowest values, in carotenoid content as well as in ASTA, which are the opposite to the values found in M1. Carotenoid values found in this study are within the ranges reported by KIMS et al. (2002), Topuz & Ozdemir (2007), Howard et al. (2000) for *C. annuum* cultivars. Except M7 and M10 which had valor ASTA lower slightly than 100, the rest are considered acceptable (ASTA 1999). High quality and expensive paprika powders usually show ASTA values of above 100. As a rule, when the ASTA color is higher, the paprika is more expensive. The ASTA color is strongly correlated with the carotenoid pigment content (Perez-Galvez et al. 2004). Peppers are a good source of carotenoids, which can vary in composition and concentration owing to differences in genetics and maturation (Markus et al. 1999; Russo & Howard 2002).

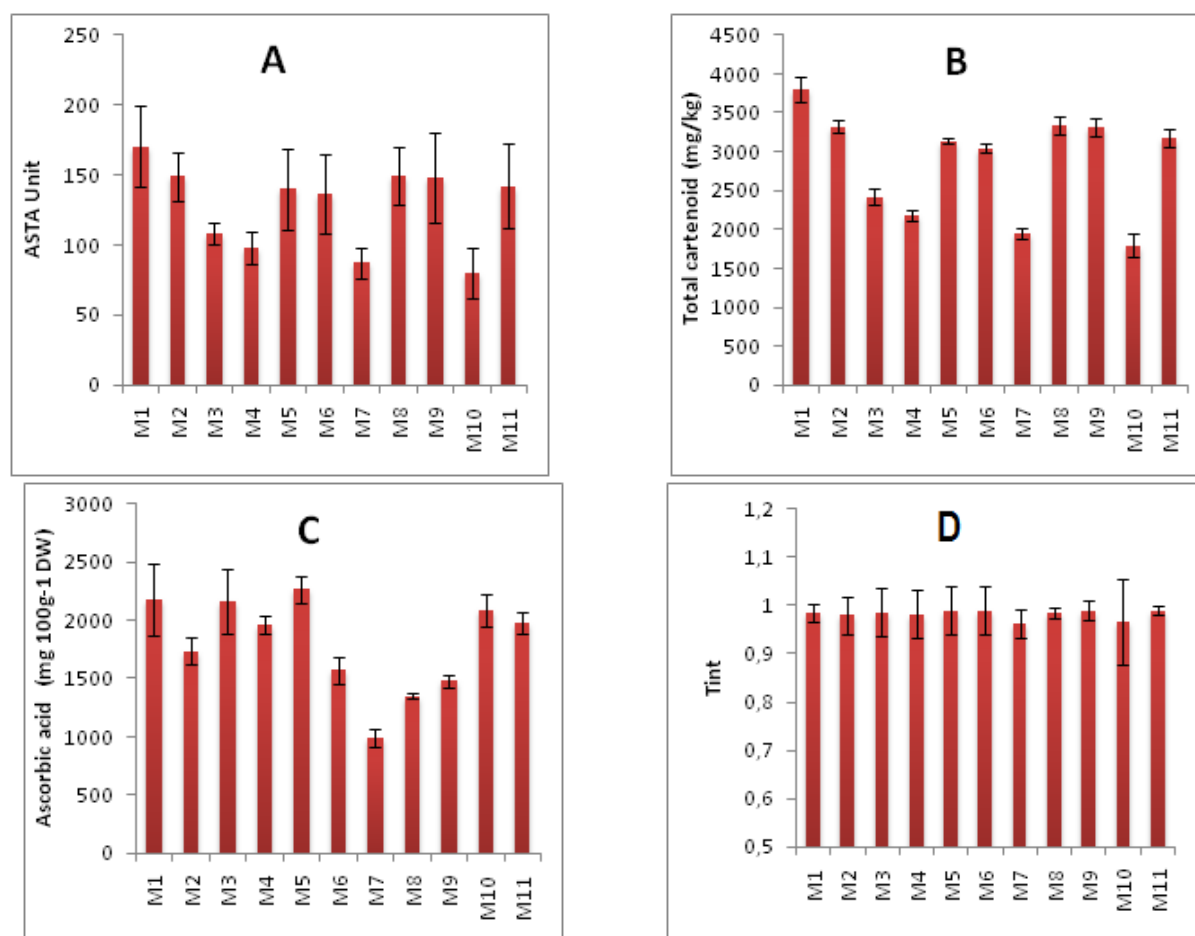


Figure 2: ASTA values (A), Carotenoid content (B), Ascorbic acid content (C) and tint (D) of the 11 morphotypes collected from Tadla-Azilal area.

3.3. Chemical composition of paprika

Vitamin C (Fig 2C) was also found to vary significantly ($P < 0.005$) among paprika morphotype, with values ranging from 992 to 2180 mg 100 g-1 dw. The maximum vitamin C content was noticed in M1, M3, M5 and the minimum in M7. The vitamin C content found in this study agrees with the values obtained by Kim et al. (2011) (1987 mg 100 g-1 dry weight). Deepa et al. (2007) reported that sweet pepper genotypes harvested at the red stage used for paprika processing may show values that range from 647 to 2135 mg 100 g-1 DW. As other studies have shown, the content of vitamin C in *C. annum* is dependent on the varieties and the maturity stage of the fruits (Khadi et al. 1987; Howard et al. 2000).

Pungency or the hot taste of pepper fruits is attributed mainly to capsaicinoid concentration. In this study, three compounds of capsaicinoid (capsaicin (CAP), dihydrocapsaicin (DH) and norhydrocapsaicin (NDH)) were analyzed (Table 3). The capsaicin is the most abundant capsaicinoids. Its concentration was higher than dihydrocapsaicin and norhydrocapsaicin in all morphotypes.

Considering total capsaicinoid content, the eleven morphotypes presented different patterns levels. Fruits from M1, M5, M6 and M7 not showed detectable levels. M2, M3, M4 and M10 had low capsaicinoid content (15-96 mg kg-1 dw). M9 and M8 had high content of capsaicinoid (2525 and 8583 mg kg-1 dw, respectively). The capsaicinoids levels observed in those morphotypes were in line with those reported by Gahungu et al. (2011) and Topuz & Ozdemir (2007).

The present morphotypes were also compared with the value of SHU, which was estimated by the method of Todd et al. (1977). The SHU values of the cultivars changed between 223.14 and 122238.76. Naturally, the cultivars which had higher capsaicin and dihydrocapsaicin contents result in higher SHU values. Consequently, the SHU value of the morphotypes M8 and M9 were higher than those of other morphotypes. Likewise, the lowest SHU value was calculated in the morphotypes M2, M3, M4 and M10, and attributed to their low content of the capsaicinoids.

Table 3: Capsaicinoids content and scoville Heat Unit (SHU) in powder obtained from fruit morphotypes

Morphotypes	Capsaicine (mg kg ⁻¹ DW)	Dihydrocapsaicin (mg kg ⁻¹ DW)	Nordihydrocapsaicin (mg kg ⁻¹ DW)	Total Capsaicinoids (mg kg ⁻¹ DW)	SHU
M1	nd	nd	nd	nd	nd
M2	32±1.25 ^d	27±2.32 ^d	17.6±1.04 ^d	76.6±1.75 ^d	1113.58±22.19 ^d
M3	12.1±0.29 ^e	8.7±0.35 ^e	11.5±0.98 ^e	32.3±0.69 ^e	441.83±18.27 ^e
M4	9.2±0.76 ^e	3.1±0.46 ^e	2.7±0.36 ^e	15±0.94 ^e	223.14±6.82 ^e
M5	nd	nd	nd	nd	nd
M6	nd	nd	nd	nd	nd
M7	nd	nd	nd	nd	nd
M8	4008.2±32.54 ^a	2229.3±13.21 ^a	2345.7±12.89 ^a	8583.2±46.65 ^a	122238.76±65.87 ^a
M9	2493± 10.54 ^b	19.2±0.24 ^b	13.2±0.76 ^b	2525.4±10.63 ^b	40569.18±13.46 ^b
M10	36.7±3.28 ^d	30.6±1.49 ^d	28.5±1.98 ^d	95.8±2.84 ^d	1348.58±22.26 ^d
M11	315±14.27 ^c	178±6.87 ^c	187±3.89 ^c	680.4±14.69 ^c	9676.4±32.47 ^c

nd. : not detected

Values are mean ± SD of 3 replicates. Means within the same row carrying different superscript letter were significantly different at $p < 0.05$ according to a Duncan multiple range test

The presence of capsaicin, dihydrocapsaicin and norhydrocapsaicin shows their maximum contribution in pungency of the morphotypes (Table 3). Capsaicin, dihydro-capsaicin and nordihydrocapsaicin were also reported to be the major capsaicinoids in Capsicum by Gnaifed et al. (2001). The literature indicates that capsaicinoids content of pepper fruits varied according to ontogenetic variety and to the ecological conditions of its habitat (Bosland 1994; Hundal & Khanna 2002). The use of SHU is the traditional method to evaluate paprika pungency (Collins et al. 1995). There are five pungency levels classified using Scoville Heat Units (SHU): non-pungent (0- 700 SHU), mildly pungent (700-3,000 SHU), moderately pungent (3000-25,000 SHU), highly pungent (25,000-70,000 SHU) and very highly pungent (> 80,000 SHU) (Weiss, 2002). Referring to this scale, M1, M3, M4, M5, M6, and M7 were classified as non-pungent; M2 and M10 as mildly pungent and M8, M9 and M11 as moderately pungent.

3. Conclusion

Morphotypes of paprika studied here differed in fruits morphology, color and chemical composition. Considering the data obtained from this study, morphotype M1 was considered the most appropriate for the development of food industries, demonstrating the high potential for more desired commercial traits such as color, pungency and DW/FW ratio. Morphotype M8 is suggested for the pharmaceutical industries for its high capsaicin yield under ecological conditions found in the region of Morocco. In the other hand the results showed that the content of the metabolites analyzed vary greatly among fruits of the 11 different morphotypes, demonstrating the potential of the current germplasm collection for genetic improvement of metabolic traits. The current findings are essential as new information to the scientific database and it may contribute to the breeders in cultivar development not only in terms of their yield, cultivation cycle, production in cool area and resistance for disease and pests, but also in the nutritional and functional composition of the product. Genetic improvement and further studies on the agronomy of these morphotypes are recommended so as to encourage its cultivation.

Acknowledgements

Authors are thankful to the University of Sultan Moulay Slimane of Morocco for their financial support. We thank Jamal Koubali, Professor at the University Sultan Moulay Slimane for reading this paper.

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